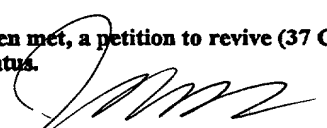


JC07 Rec'd PCT/PTO 13 FEB 2002

FORM PTO-1390 (REV. 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 30394-1064	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/049579	
INTERNATIONAL APPLICATION NO. PCT/NL00/00559		INTERNATIONAL FILING DATE 09 August 2000 (09.08.2000)		PRIORITY DATE CLAIMED 13 August 1999 (13.08.1999)	
TITLE OF INVENTION PHARMACEUTICAL PRODUCT FOR THE TREATMENT OF VIRAL INFECTIONS, IN PARTICULAR OF THE HUMAN IMMUNODEFICIENCY VIRUS (HIV)					
APPLICANT(S) FOR DO/EO/US VAN ASBECK, Bernt Sweder; MARX, Johannes Josephus Maria					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.</p> <p>4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p style="margin-left: 20px;">a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</p> <p style="margin-left: 20px;">b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</p> <p style="margin-left: 20px;">c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</p> <p>6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</p> <p style="margin-left: 20px;">a. <input type="checkbox"/> is attached hereto.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p style="margin-left: 20px;">a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> have been communicated by the International Bureau.</p> <p style="margin-left: 20px;">c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p style="margin-left: 20px;">d. <input checked="" type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).</p> <p>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). [UNSIGNED]</p> <p>10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11 to 20 below concern document(s) or information included:</p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input type="checkbox"/> A FIRST preliminary amendment.</p> <p>14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>15. <input type="checkbox"/> A substitute specification.</p> <p>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.</p> <p>18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</p> <p>19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</p> <p>20. <input checked="" type="checkbox"/> Other items or information: Associate Power of Attorney; Small Entity Status is claimed.</p>					

U.S. APPLICATION NO. (37 CFR 1.53) 10/049579		INTERNATIONAL APPLICATION NO. PCT/NL00/00559		ATTORNEY'S DOCKET NUMBER 30394-1064	
21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	6 - 20 =	0	x \$18.00	\$	0
Independent claims	1 - 3 =	0	x \$80.00	\$	0
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$	890.00
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+	\$ (445.00)
SUBTOTAL =				\$	445.00
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	445.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				\$	445.00
				Amount to be refunded:	\$
				charged:	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>445.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>13-4213</u> . A duplicate copy of this sheet is enclosed. d. <input type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: MYERS, Jeffrey D. PEACOCK, MYERS & ADAMS, P.C. P.O. Box 26927 Albuquerque, New Mexico 87125-6927 US					
				 SIGNATURE	
				MYERS, Jeffrey D. NAME	
				35,964 REGISTRATION NUMBER	

Pharmaceutical product for the treatment of viral infections, in particular of the human immunodeficiency virus (HIV)

The present invention relates to a pharmaceutical product for the treatment of viral infections, in particular of the human immunodeficiency virus (HIV).

5 Viral infections, in particular infections by the human immunodeficiency virus (HIV) are the serious threat to public health. World-wide much effort goes into the development of a pharmaceutical product to combat this usually incurable disease.

10 It is known that iron is involved in the replication of human immunodeficiency virus. In the first place, the HIV-1 long terminal repeat (LTR) portion contains nuclear factor (NF)-kB response elements that regulate proviral transcription. NF-kB activation may be influenced with iron via the production of reactive oxygen
15 species.

A second route by which iron chelation can influence HIV replication is by inhibition of the DNA synthesis by inactivation of iron-dependent ribonucleotide reductase.

20 Another strategy which can be employed in targeting iron chelators against HIV-1 is a direct viral DNA/RNA attack.

It is the object of the present invention to provide a pharmaceutical product for the efficient
25 treatment of viral infection, in particular of the human immunodeficiency virus, to which end the pharmaceutical product is characterized in that the pharmaceutical product comprises a compound that as active component contains an iron chelator.

30 Suitable iron chelators according to the invention may be selected from the group of hydroxamates (such as deferoxamine), the family of the

hydroxypyridinons (such as deferiprone), and the nucleic acid-binding chemotherapeutical products (such as bleomycin).

Very surprisingly it has been shown that in vitro particularly favourable results were obtained when in addition to the component containing as the active compound an iron chelator, a preparation containing another virus-inhibiting compound was used; which was due to a synergistic effect. A protease inhibitor, in particular ritonavir, proved to be especially suitable as the antiviral agent.

Another antiviral agent producing a favourable synergistic effect with iron chelators is reverse transcriptase inhibitor, in particular dideoxyinosine.

In this connection applicant has examined the effect of deferoxamine (DF), deferiprone (L1) and bleomycin (BLM) on HIV-1 replication in primary blood cells. For this purpose human peripheral blood lymphocytes (PBL) and monocyte-derived macrophages (MDM), the main target cells for HIV, were used. It was shown, that the considerable production of HIV-1 replication by DF was accompanied by a proliferation inhibition. The same was observed with L1. Surprisingly, BLM inhibited the viral replication in PBL and MDM without affecting lymphocyte proliferation. The use of iron chelators in combination with established antiviral agents for antiviral combination therapy was examined. When DF was used in combination with the nucleoside analog dideoxyinosine (ddI), a surprising synergistic inhibition of p24 in HIV-infected PBL seemed to occur.

Materials and methods used in the study are the following.

Cell isolation.

Peripheral Blood Mononuclear Cell (PBMC) fractions were isolated from heparinized blood from HIV-1-, HIV-2- and hepatitis B-seronegative donors (Bloodbank, Utrecht, The Netherlands) by Ficoll-Isopaque

gradient separation. Cells were washed twice and were then either subjected to counter-current centrifugal elutriation, or monocytes in the PBMC fraction were allowed to adhere to fibronectin-coated flasks before the PBL fraction was harvested. Monocytes were of >95% purity by criteria of cell morphology on May-Grünwald-Giemsa-stained cytosmears and by non-specific esterase staining using (α -naphthylacetate (Sigma Chemical Co., St. Louis, MO) and PBL >85% purity by May-Grünwald-Giemsa-staining. Viability of both cell types was >95% at the point of experiment initiation as determined by trypan-blue exclusion. Isolated monocytes were cultured in suspension at a concentration of 2×10^6 cells/ml in teflon flasks minimizing adherence (Nalgene, Rochester, NY) and allowed to differentiate for 7 days. MDM medium consisted of Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated human AB serum, negative for anti-HIV antibodies (Bloodbank Utrecht, NL), 10 μ g/ml gentamycin (Life Technologies Ltd, Paisley, Scotland), 5 μ g/ml ciprofloxacin (Bayer, Leverkusen, Germany), 0.6 mg/ml L-glutamine (Sigma) and 3.4 g/L bicarbonate (Merck, Darmstadt, Germany). Isolated PBL was stimulated to proliferate for 3 days with 4 μ g/ml phytohemagglutinin (PHA; Sigma) in RPMI-1640 (Life Technologies Ltd.) medium supplemented with 10% heat inactivated fetal calf serum (Life Technologies Ltd.) and 10 μ g/ml gentamycin. After PHA stimulation the PBL were cultured in medium containing 10 U/ml human recombinant IL-2 (Boehringer, Mannheim, Germany). All incubations were carried out in flat-bottomed 96-well plates at 37 °C, 5% CO₂ and 95% air.

HIV infection and p24 measurements.

Cells were infected for 2 hours with HIV-1_{BR-L}. Macrophages were infected with a multiplicity of infection (MOI) of 0.005 and lymphocytes with an MOI of 0.001. The cells were then washed twice to remove excess virus and subsequently incubated with either DF (Novartis Pharma, Arnhem, The Netherlands), L1 (from Duchefa Farma B.V.,

Haarlem, The Netherlands) or BLM (H. Lundbeck A/S, Copenhagen, Denmark) for a period of 5 days. Virus in culture supernatant was inactivated in a final concentration of 0.05% empigen (Calbiochem-Novabiochem Co., La Jolla, CA). The presence of HIV-1 in the inactivated supernatant was monitored by checking for the p24-core antigen by enzyme-linked immunosorbent assay (ELISA).

Lymphocyte proliferation measurements.

Since iron chelators may influence cellular proliferation the effect of the three iron chelators on PBL proliferation was also determined. This was performed by monitoring overnight ³H-thymidine (³H-TdR) incorporation into DNA. 25 µl ³H-TdR (0.5 µCi, Amersham International plc, UK) was added in the presence of DF, L1, BLM or DF/ddI combination, in a total volume of 200 µl cell suspension after 4 days incubation with the different compound concentrations. Cell incubations were in 96-well plastic plates (Costar, Cambridge, MA, US). After overnight incubation with ³H-TdR, the cells were harvested onto a filter using an automatic cell microharvester (Titertek® cell harvester 530). The filter was subsequently dried and immersed in a scintillation cocktail (Wallac UK). Radioactivity was counted in a Mark II liquid Scintillation system (Model x, Tricton analytics, Inc.).

Cytotoxicity.

Possible cytotoxicity of DF, L1 or BLM was monitored by the MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma) assay.

Studies of DF-ddI combination.

Three separate experiments were performed substantially as described in the section *HIV infection and p24 measurements*. Multiply-diluted fixed-ratio combinations of the drugs, or single drugs were added to

each incubation point. The IC_{50} (inhibitory concentration) of each drug was calculated using the computer software program CalcuSyn. The combined-drug effect was evaluated by the median-effect principle and the isobologram method.

5 This method involves the plotting of the dose-effect curve for each drug alone and in combination. The slope of the median-effect plot and the x intercept of the plot were then used for the computerized calculation of a combination index (CI). The CI values were based on the classic isobologram equation and CI values of <1 , 1 and >1 indicate synergism (greater than the expected additive effect), additive effects and antagonism (less than the expected additive effect). Fraction affected (f_a) was calculated for every drug combination and for each drug alone after p24 measurements in infected PBL incubated for 15 5 days with the examined compounds (see *HIV infection and p24 measurements*).

Statistical analysis.

20 Repeated measures ANOVA and student Newman-Keuls test were used to analyze the data. P values below 0.05 were considered significant.

Results

25 *Effect of DF on HIV-1 replication, cellular proliferation and cytotoxicity in PBL.*

DF was shown to reduce p24 levels in culture supernatants. At a concentration of $3 \mu M$ DF a 40% reduction was observed in viral replication in PBL (19 vs 30 11 ng p24/ml, $n=3$, $p<0.05$). A compound concentration of $30 \mu M$ reduced p24 levels by $>90\%$ (figure IA) from 19 to 1.9 ng p24/ml ($n=3$). To rule out the possibility that these inhibitory effects were due to cytotoxicity, cell viability was monitored. A DF concentration of $100 \mu M$ was found to be cytotoxic (figure IB). In addition, due to the 35 anti-proliferative properties of DF, cellular proliferation was monitored. The observed reduction in p24 was mirrored by a decrease in proliferation (figure IC).

Thus, non-cytotoxic DF concentrations were capable of reducing HIV replication and this reduction was accompanied by cellular proliferation inhibition.

5 *Effect of L1 on HIV-1 replication, cellular proliferation and cytotoxicity in PBL.*

10 HIV-1 replication in lymphocytes was reduced to minimal levels by 100 μ M L1 (figure 2A). This concentration was not cytotoxic in PBL (figure 2B). There seemed to be a threshold point in L1 concentration where the inhibition slope became very steep and this phenomenon was also mirrored by a decrease in proliferation (figure 2C). Thus, the HIV-inhibition observed with L1 could also be attributed to cellular proliferation inhibition. At
15 lower compound concentrations a subtle increase in p24 antigen production was observed (figure 2A).

20 *Effect of BLM on HIV-1 replication, cellular proliferation and cytotoxicity in PBL.*

20 BLM dose-dependently decreased HIV-1 replication in lymphocytes (figure 3A). This compound was not cytotoxic in PBL (figure 3B). At concentrations in the range of 3-300 ng BLM/ml a 20-50% reduction was noted, reflecting a decrease from 19 to 15 (20%) or 9.5 (50%) ng
25 p24/ml (n=3) (figure 3A). Cellular proliferation remained intact (figure 3C). Thus, HIV-1 inhibition by BLM, in contrast to DF and L1, seems to proceed via a pathway other than cellular proliferation inhibition.

30 *Effect of DF, L1, and BLM on HIV-1 replication and cytotoxicity in MDM.*

35 In MDM, DF concentrations around 100 μ M reduced viral replication by up to 50% (1.2 vs 0.6 ng p24/ml) in a dose-dependent manner (figure 4AI). However this p24 decrease was associated with the onset of cytotoxicity (figure 4AII). L1 in the concentrations studied, strikingly up-regulated p24 antigen production by up to

two fold at 30 μM (figure 4BI). These concentrations were not cytotoxic (figure 4BII).

In MDM a dose-dependent decrease in p24 levels was also observed with a 50% reduction at 300 ng BLM/ml from 1.2 to 0.6 ng p24/ml (figure 4CI). BLM concentrations, similarly to PBL, were found not to affect cell viability in MDM (figure 4CII).

Combined antiviral effects of DF-ddI.

Moderate to strong synergism was detected when DF was used in combination with ddI. Figure 5 shows a representative graph of the combination index (CI) with respect to fraction affected (f_a) for the inhibition of HIV by a mixture of DF and ddI (molar ratio 4:1). IC_{50} of DF was 8.5 μM and that of ddI 3.7 μM . Figure 5 clearly shows that there is a marked synergism at all f_a values obtained with the different combinations, with the synergism getting stronger as f_a increases. At around the IC_{50} concentrations of both DF and ddI (combination points on figure 5: 10 μM DF: 2.5 μM ddI), the highest f_a value was obtained and the combination was strongly synergistic. Cellular proliferation was also monitored with these combinations and with each drug alone. It was found that ddI in combination with DF did not have an additional inhibitory effect on cellular proliferation when compared to DF alone (results not shown).

The invention is explained in more detail with reference to the Figures 1-6.

Fig. 1. Effect of DF on HIV-I replication (A), cytotoxicity (B) and cellular proliferation (C) in PBL after 5 days incubation with DF.

For the HIV-1 replication measurements cells were infected for 2 hours with HIV-1_{89.6} at an MOI of 0.001; the cells were subsequently washed 2 times and incubated with different DF concentrations. After 5 days, supernatant was harvested for p24 ELISA. Virus replication was expressed as % p24 reduction after DF incubations, compared to p24 measured from control HIV-infected cells incubated in the

absence of DF (set at 100% p24). Cytotoxicity was measured by the MTT test and is represented as % viable cells after 5 days DF incubations, compared to viable cells from control HIV-infected cells incubated in the absence of DF (set at 100% viable cells). PBL proliferation was measured by overnight ³H-thymidine incorporation after DF was incubated for 4 days with cells; it is represented as counts per minute (cpm) after DF incubations, compared to cpm from control cells, incubated in the absence of DF.

Results are the average of 3 experiments in duplicate (statistical analysis using repeated ANOVA and student Newman-Keuls test, *p<0.05). 100% p24 production represents 19 ng p24/ml

Fig. 2. Effect of L1 on HIV-1 replication (A), cytotoxicity (B) and cellular proliferation (C) in PBL after 5 days incubation with L1.

For the HIV-I replication measurements cells were infected for 2 hours with HIV-1_{Ba-L} at an MOI of 0.001; the cells were subsequently washed 2 times and incubated with different L1 concentrations. After 5 days, supernatant was harvested for p24 ELISA. Virus replication was expressed as % p24 reduction after L1 incubation, compared to p24 measured from control HIV-infected cells incubated in the absence of L1 (set at 100% p24). Cytotoxicity was measured by the MTT test and is represented as % viable cells after 5 days L1 incubations, compared to viable cells from control HIV-infected cells incubated in the absence of L1 (set at 100% viable cells). PBL proliferation was measured by overnight ³H-thymidine incorporation after L1 was incubated for 4 days with cells; it is represented as counts per minute (cpm) after L1 incubations, compared to cpm from control cells, incubated in the absence of L1. Results are the average of 3 experiments in duplicate (statistical analysis using repeated ANOVA and student Newman-Keuls test, *p<0.05). 100% p24 production represents 19 ng p24/ml.

Fig. 3. Effect of BLM on HIV-1 replication (A), cytotoxicity (B) and cellular proliferation (C) in PBL after 5 days incubation with BLM.

For the HIV-1 replication measurements cells were infected for 2 hours with HIV-1_{8a-L} at an MOI of 0.001; the cells were subsequently washed 2 times and incubated with different BLM concentrations. After 5 days, supernatant was harvested for p24 ELISA. Virus replication was expressed as % p24 reduction after BLM incubations, compared to p24 measured from control HIV-infected cells incubated in the absence of BLM (set at 100% p24). Cytotoxicity was measured by the MTT test and is represented as % viable cells after 5 days BLM incubations, compared to viable cells from control HIV-infected cells incubated in the absence of BLM (set at 100% viable cells). PBL proliferation was measured by overnight ³H-thymidine incorporation after BLM was incubated for 4 days with cells; it is represented as counts per minute (cpm) after BLM incubations, compared to cpm from control cells, incubated in the absence of BLM. Results are the average of 3 experiments in duplicate (statistical analysis using repeated ANOVA and student Newman-Keuls test, *p<0.05). 100% p24 production represents 19 ng p24/ml

Fig. 4. Effect of DF (AI-II), L1 (BI-II) and BLM (CI-II) on HIV-1 replication (A-CI) and cytotoxicity (A-CII) in MDM after 5 days incubation.

For the HIV-1 replication measurements cells were infected for 2 hours with HIV-1_{8a-L} at an MOI of 0.001; the cells were subsequently washed 2 times and incubated with different compound concentrations. After 5 days supernatant was harvested for p24 ELISA; Virus replication was expressed as % p24 reduction after compound incubations, compared to p24 measured from control HIV-infected cells incubated in the absence of compounds (set at 100% p24). Cytotoxicity was measured by the MTT test and is represented as % viable cells after 5 days compound incubations, compared to viable cells from control HIV-

infected cells incubated in the absence of compounds (set at 100% viable cells). Results are the average of 3 experiments in duplicate (statistical analysis using repeated ANOVA and student Newman-Keuls test, $*p < 0.05$).

5 100% p24 production represents 1.2 ng p24/ml.

Fig. 5. Graphical presentation of the combination index (CI) with respect to fraction affected (fa) for the p24-antigen inhibition by a mixture of DF and ddI (molar ratio 4:1). Data points on the graph are indicated as conc
10 DF and ddI, both in μM . $\text{CI} < 1$, $= 1$ and > 1 represent synergistic, additive and antagonistic effects, respectively. The subdivision within the synergistic area ($\text{CI} > 0.7$, $0.3-0.7$ and < 0.3 implying moderate synergism, synergism, and strong synergism respectively) is according
15 to Chou and Talalay. The parameters are obtained using the median effect equation of Chou and Talalay and the CalcuSync computer software programme as described in materials and methods. The IC_{50} within this experiment of DF was $8.5 \mu\text{M}$ and that of ddI was $3.7 \mu\text{M}$. This graph is
20 representative of 3 independent experiments.

Fig. 6. Graphical presentation of the combination index (CI) with respect to fraction affected (fa) for the p24-antigen inhibition by a mixture of bleomycin (BLM) and ritonavir (ratio 30:1). Peripheral blood lymphocytes (PBL)
25 were infected for 2 hours with HIV-1_{Ba-L} at an MOI of 0.001. The cells were subsequently washed 2 times to remove an excess in virus and subsequently incubated for 5 days with only BLM, only ritonavir or various combinations of the two substances. Virus in the supernatant of the cell
30 cultures was inactivated in a final concentration of 0.05% empigen and the presence of HIV-1 in the inactivated supernatant was measured by determining the p24 antigen with enzyme-linked immunosorbent assay (ELISA). $\text{CI} < 1$, $= 1$ and > 1 indicate synergistic, additive and antagonistic
35 effects, respectively, according to Chou and Talalay. The parameters are obtained using the median effect equation of Chou and Talalay, and the CalcuSync computer software programme. In this experiment the IC_{50} of BLM was $1.9 \mu\text{g/ml}$

and of ritonavir 0.04 μM . This graph is representative of two independent experiments.

NEW CLAIMS

1. A pharmaceutical product for the treatment of viral infections, in particular of the human immunodeficiency virus (HIV), **characterized** in that the pharmaceutical product comprises a compound that as active component contains an iron chelator and a component comprising another virus-inhibiting compound.

2. A pharmaceutical product according to claim 1, **characterized** in that the iron chelator is selected from the group of hydroxamates (such as deferoxamine), the family of the hydroxypyridinons (such as deferiprone), and the nucleic acid-binding chemotherapeutical products (such as bleomycine).

3. A pharmaceutical product according to claim 2, **characterized** in that the virus-inhibiting compound is a protease-inhibitor.

4. A pharmaceutical product according to claim 3, **characterized** in that the protease-inhibitor is ritonavir.

5. A pharmaceutical product according to claim 1, **characterized** in that the virus-inhibiting compound is a reverse transcriptase inhibitor.

6. A pharmaceutical product according to claim 5, **characterized** in that the reverse transcriptase inhibitor is a dideoxyinosine.

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(72) Inventors; and

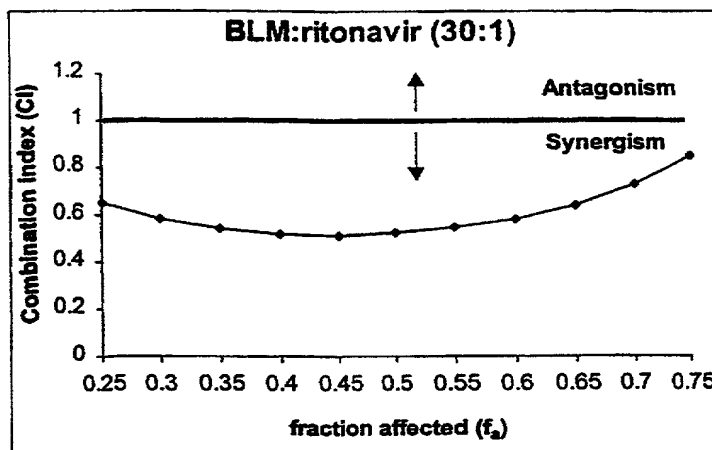
(75) Inventors/Applicants (*for US only*): **VAN ASBECK, Bernt, Sweder** [NL/NL]; Verheullaan 19, NL-3971 RD Driebergen (NL). **MARX, Johannes, Josephus, Maria**

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[Continued on next page]

(54) Title: PHARMACEUTICAL PRODUCT FOR THE TREATMENT OF VIRAL INFECTIONS, IN PARTICULAR OF THE HUMAN IMMUNODEFICIENCY VIRUS (HIV)



(57) Abstract: The invention describes a pharmaceutical product for the treatment of viral infections, in particular of the human immunodeficiency virus (HIV). According to the invention, the pharmaceutical product comprises a compound that as active component contains an iron chelator. Suitable iron chelators are selected from the group of hydroxamates (such as deferoxamine), the family of the hydroxypyridinons (such as deferiprone), and the nucleic acid-binding chemotherapeutical products (such as bleomycine). A synergistic effect is obtained in vitro when in addition to the component containing the iron chelator, a product is used that contains another virus-inhibiting compound. As the antiviral agent a protease-inhibitor, preferably ritonavir, is considered. Another suitable antiviral agent is a reverse transcriptase inhibitor, preferably a dideoxyinosine.

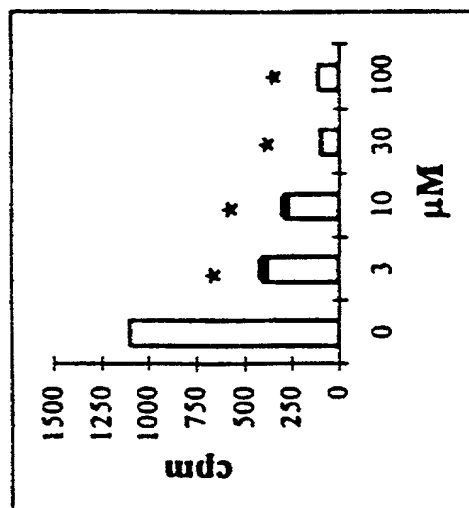
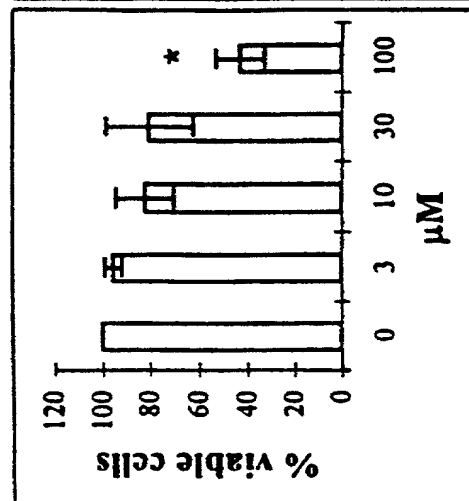
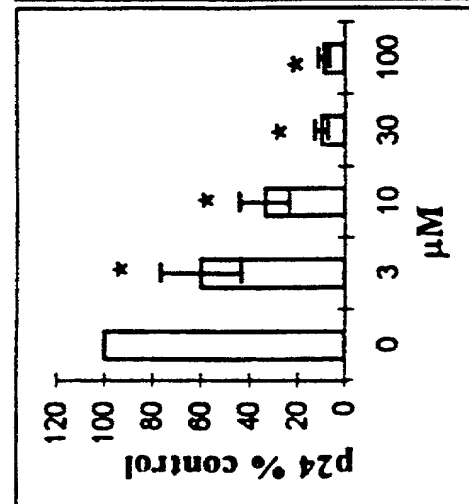
C: proliferation**B: viable cell count****A: p24 antigen**

FIG. 1

10/049579

A: p24 antigen **B: viable cell count** **C: proliferation**

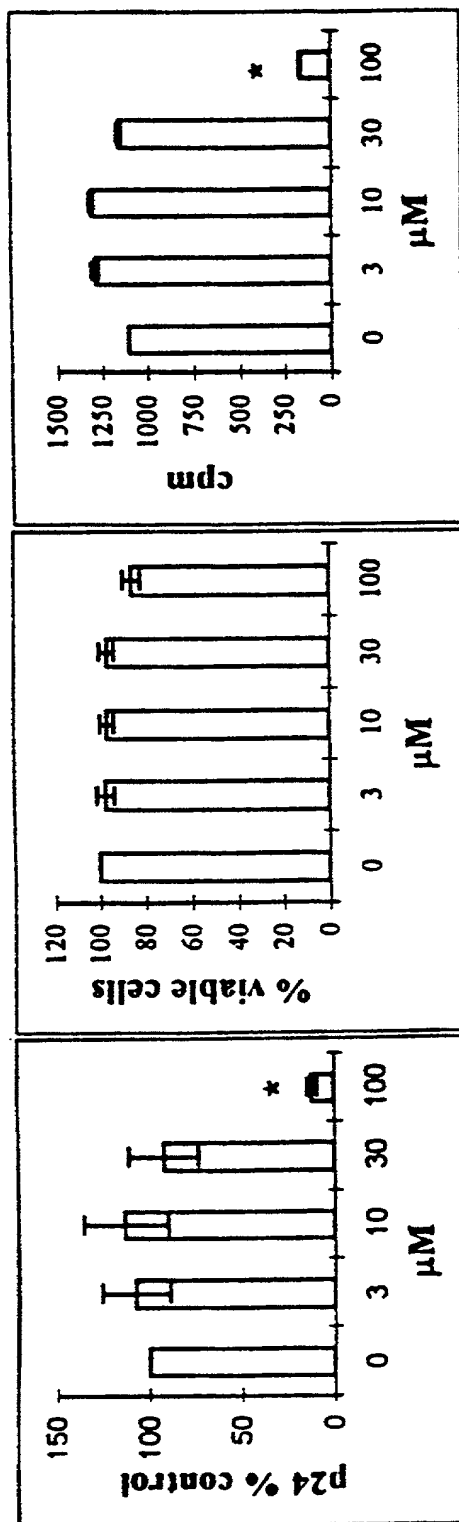


FIG. 2

A: p24 antigen **B: viable cell count** **C: proliferation**

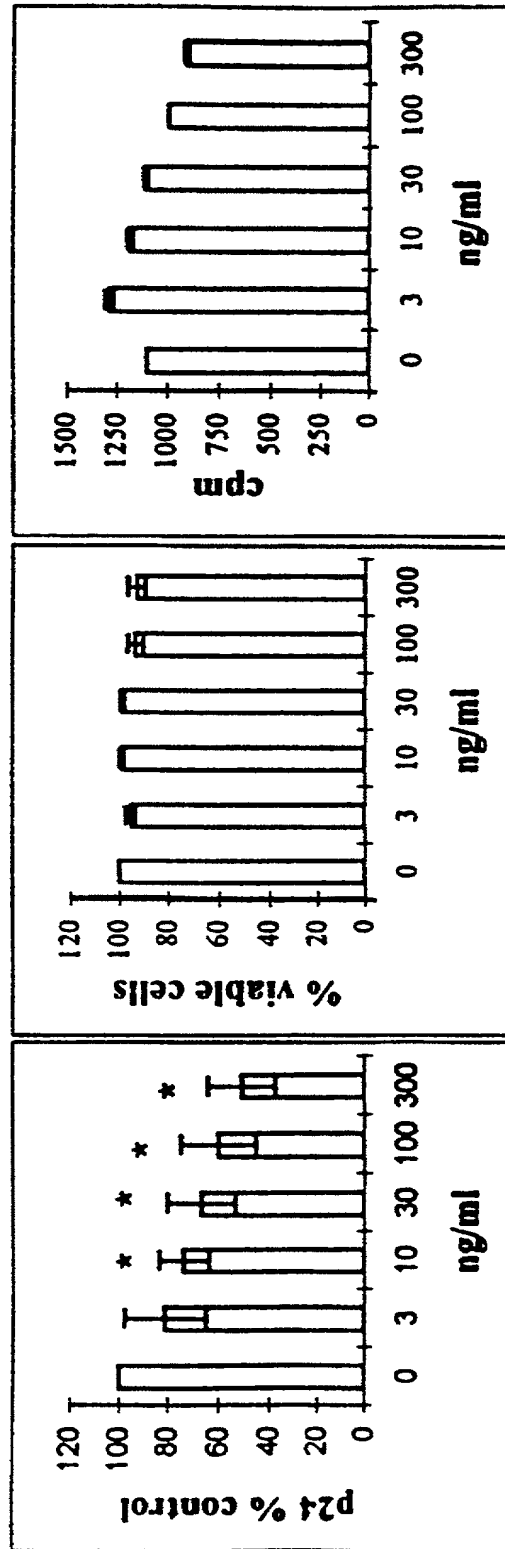


FIG. 3

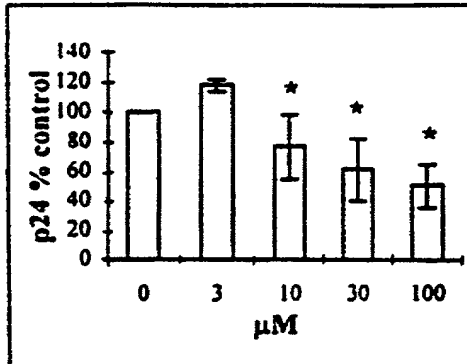
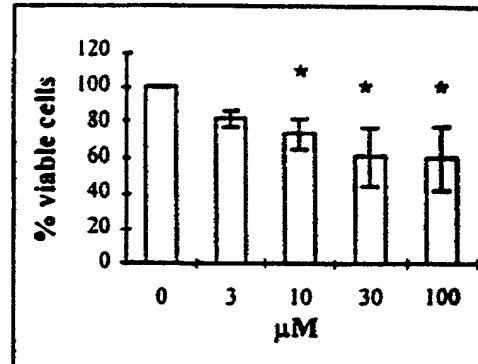
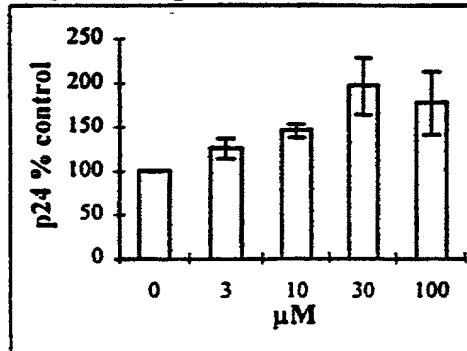
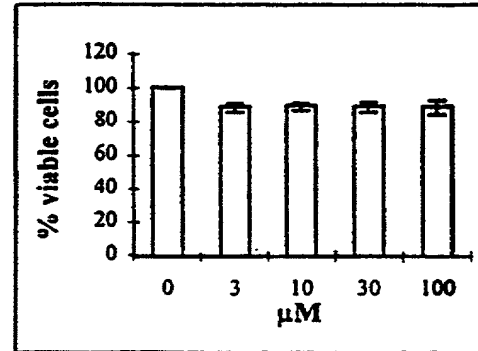
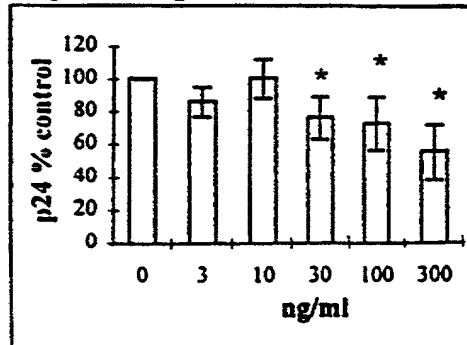
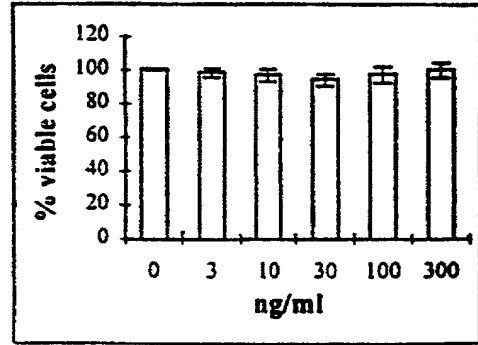
A: Deferoxamine (DF)**I: p24 antigen****II: viable cell count****B: Deferiprone (L1)****I: p24 antigen****II: viable cell count****C: Bleomycin (BLM)****I: p24 antigen****II: viable cell count**

FIG. 4

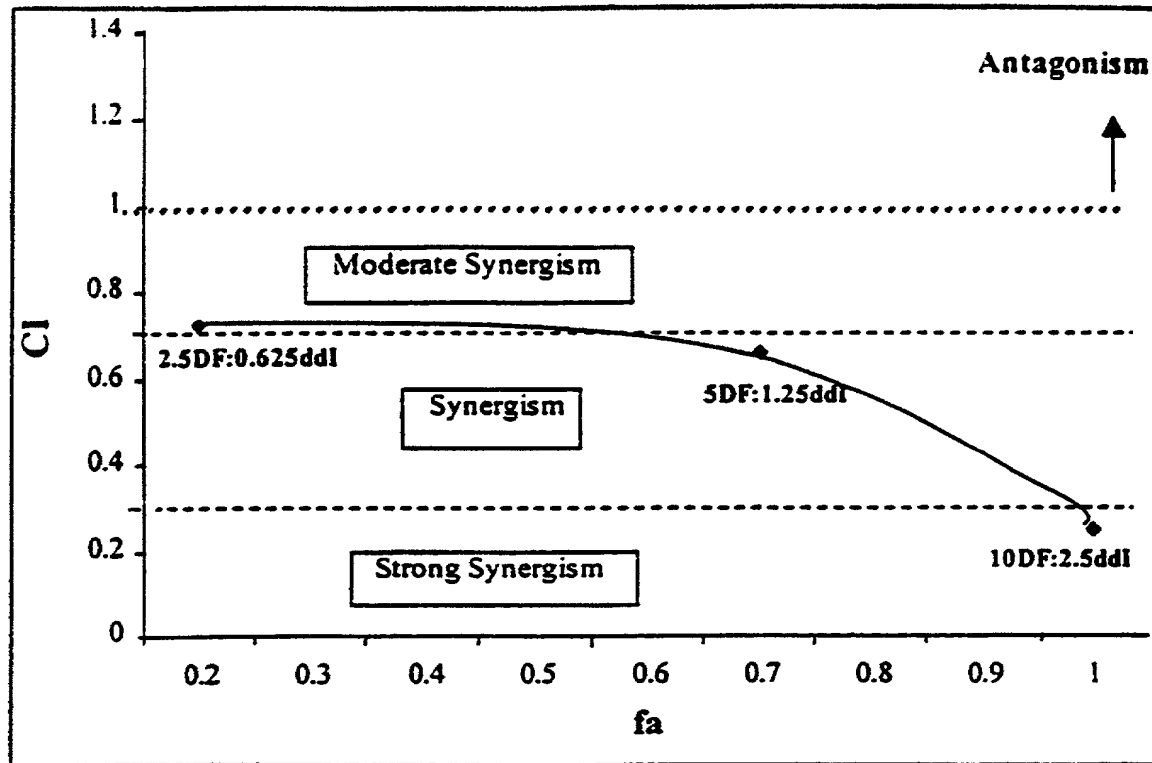


FIG. 5

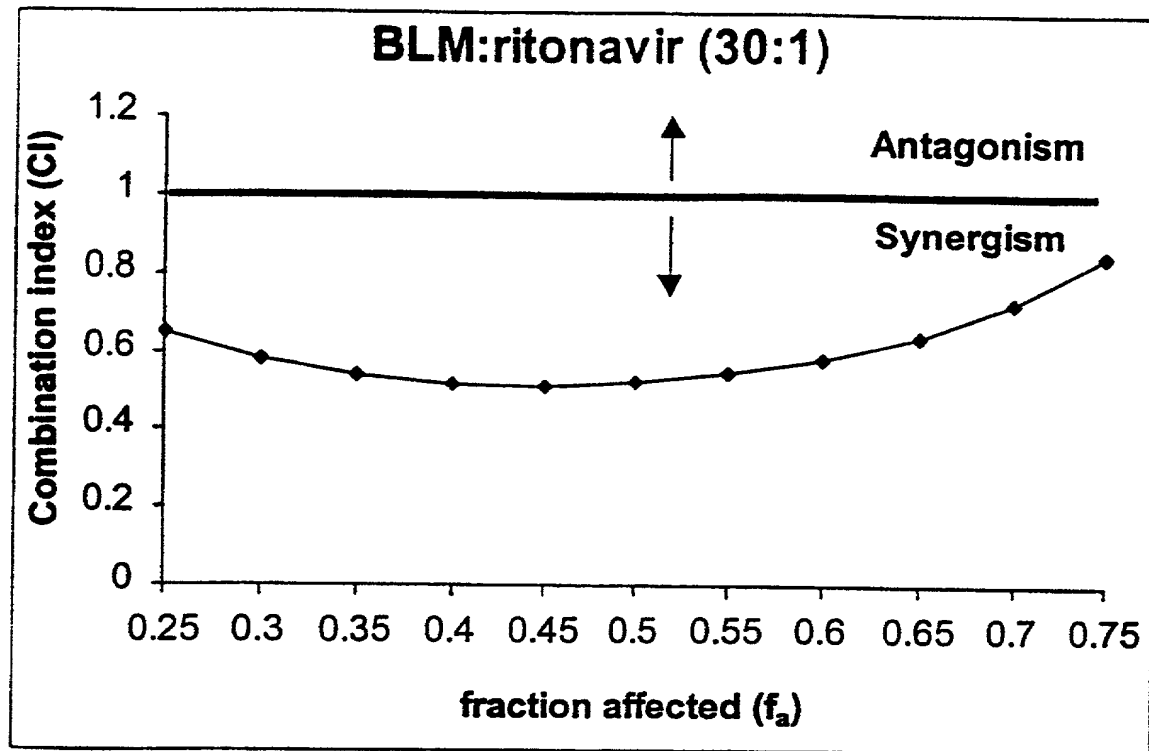


FIG. 6

PATENT APPLICATION

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Diane S. Nelson, Paralegal

13 February 2002
Date Signed

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): VAN ASBECK, Bernt Sweder, et al. :
Serial No.: UNKNOWN : Attorney Docket No.: 30394-1064
Filed: 13 February 2002 : Anticipated Group Art Unit: UNKNOWN
For: PHARMACEUTICAL PRODUCT FOR THE :
TREATMENT OF VIRAL INFECTIONS, IN :
PARTICULAR OF THE HUMAN :
IMMUNODEFICIENCY VIRUS (HIV) :

ASSOCIATE POWER OF ATTORNEY

Box: PCT
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

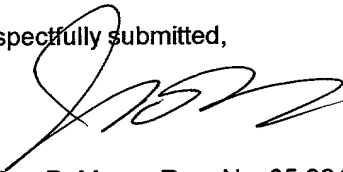
Jeffrey D. Myers, a principal attorney in the above-identified application for Letters Patent, hereby

appoints:

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Paul Adams, Reg. No. 21,096
Rod D. Baker, Reg. No. 35,434
Andrea L. Mays, Reg. No. 43,721;
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Respectfully submitted,



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ATTORNEY'S DOCKET NUMBER

(includes Reference to PCT International Applications)

30394-1064

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PHARMACEUTICAL PRODUCT FOR THE TREATMENT OF VIRAL INFECTIONS.
IN PARTICULAR OF THE HUMAN IMMUNODEFICIENCY VIRUS (HIV)

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Serial No. 10/049,579

on February 13, 2002

and was amended

on _____ (if applicable).

 was filed as PCT international application

Number PCT/NL00/00559

09 August 2000 (09.08.2000)

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
PCT	PCT/NL00/00559	09 August 2000	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
Netherlands	1012825	13 August 1999	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

Combined Declaration For Patent Application and Power of Attorney (Continued)

ATTORNEY'S DOCKET NUMBER
30394-1064

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120

U.S. APPLICATIONS			STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE		PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			

POWER OF ATTORNEY. As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)

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	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	
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	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
	<u>[Signature]</u>	<u>[Signature]</u>
DATE	DATE	DATE
	<u>10 April 2002</u>	<u>10 April 2002</u>

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(Combined Declaration For Patent Application and Power of Attorney—PTO 1391 [13-11]—page 2 of 2)